AD_____

Award Number: DAMD17-01-1-0349

TITLE: A Population-Based Investigation of the Role of Focal Adhesion Kinase (FAK) and E-cadherin Expression in Breast Cancer Promotion, Progression and Therapeutic Response

PRINCIPAL INVESTIGATOR: Sandra L. Deming

CONTRACTING ORGANIZATION: University of North Carolina
Chapel Hill, NC 27599-1350

REPORT DATE: October 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2003	Annual Summary (1 Oct 2002 - 30 Sep 2003)

4. TITLE AND SUBTITLE
A Population-Based Investigation of the Role of Focal Adhesion Kinase (FAK) and E-cadherin Expression in Breast Cancer Promotion, Progression and Therapeutic Response

5. FUNDING NUMBERS
DAMD17-01-1-0349

6. AUTHOR(S)
Sandra L. Deming

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of North Carolina
Chapel Hill, NC 27599-1350

E-Mail: deming@email.unc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Presented within this annual report are the details of the second year's progress and the preliminary results of the grant entitled "A population based investigation of the role of Focal Adhesion Kinase (FAK) and E-cadherin expression in breast cancer promotion, progression, and therapeutic response". In regards to the specific aims of the grant, immunohistochemical staining has been completed for both FAK and E-cadherin expression. Scoring has been completed on all 629 samples stained for FAK, providing information regarding the prevalence and pattern of FAK expression as well as its association with other known risk and prognostic factors for breast cancer. Scoring has been completed on all *** sample for E-cadherin following the development of a more-comprehensive scoring system. Collection of survival data is ongoing. Completing the proposed aims should elucidate the role of FAK and E-cadherin expression as a verifiable marker of disease aggressiveness, and will provide a better understanding of the biological behavior thus allowing for a more tailored treatment of breast cancer.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction:

One of the current problems in breast cancer diagnosis and treatment is the lack of verifiable markers indicative of prognosis, and therapeutic response. This is particularly important since many breast tumors are histologically similar yet behave quite differently. By identifying molecular markers that are early indicators of more aggressive disease, we can better tailor treatments to achieve optimal results. Our study proposed to investigate the role of two such markers: E-cadherin and Focal Adhesion Kinase (FAK) as tenable prognostic markers in breast cancer. To address this question, tumor tissue from phase I cases of the Carolina Breast Cancer Study (CBCS) have been immunohistochemically stained for E-cadherin and FAK expression. CBCS is a population-based study in North Carolina that includes nearly 800 cancer cases collected from 1993-96. Extensive demographic information as well as medical, exposure, and work histories are available for the participants, which will allow for the evaluation of the independent role of E-cadherin and FAK in the context of known risk and prognostic factors. Determining the potential for FAK and E-cadherin to identify breast tumors requiring more aggressive treatment at an early stage could ultimately increase survival and reduce breast cancer mortality.

Body:

Work progress with respect to the initial aims of the grant:

Aim 1:
a. Stain invasive tumor tissue sections with antibodies against FAK and E-cadherin
b. Quantify immunohistochemical staining results

Progress:
a. Both FAK and E-cadherin immunohistochemical staining have been completed for the phase I cases as proposed.
b. Quantification has been complete for FAK stained tissue (complete *n* =629).
Development of a more comprehensive scoring system has been completed for E-cadherin staining.
Scoring has been completed for all E-cadherin stained tissue (complete *n* =598)

Aim 2:
a. Statistically analyze the association between expression levels and invasive disease
b. Determine the correlation between expression levels and stage of disease

Progress:
a. Preliminary analysis has been conducted for FAK stained and quantified tissue (*n* = 629)
b. Preliminary analysis has been conducted for FAK stained and quantified tissue (*n* = 629)

Aim 3:
a. Stratify women based on race and statistically

determine if expression levels vary between African American and white breast cancer patients

b. Stratify women based on age and statistically determine if expression levels vary between younger and older breast cancer patients

Progress: a. Analysis not yet begun

b. Analysis not yet begun

Aim 4: a. Obtain updated treatment histories for participants

b. Ascertain current disease status, including recurrence and survival information for participants

c. Statistically analyze the data to evaluate the association between FAK and E-cadherin expression levels and therapeutic response and survival

Progress: a. Data currently under collection

b. Our initial plan was to collect vital status on our cases via the Social Security Administration. However, we were unable to obtain cause-specific mortality from this source. We now will obtain this information from the National Center for Health Statistics' National Death Index (NDI) as this source will be able to provide us with cause-specific mortality data for our cases. We have submitted the NDI application and are awaiting approval (expected by the end of November). We should receive cause-specific mortality data shortly following approval.

c. Not yet begun, awaiting data from NDI

Preliminary results as of October 15, 2003: FAK

Please note, the data and results presented in the document are preliminary and unpublished, and thus should be protected accordingly.

Table 1 presents the characteristics of the patients in this dataset. The average age of the cases in our dataset was 49.5 years of age and presented with a full range of different stages of disease at the time of diagnosis, which facilitated complete analysis by stage.

Table 1. Characteristics of the patients with tissue stained for FAK

N = 629	
Age (years)	
Mean	50.1
Median (Range)	48.0 (23-74)
Race *n* (%)	
African American	261 (41.5%)
Non-African American	368 (58.5%)
Stage *n* (%)	
1	227 (38.9%)
2	292 (50.0%)
3+4	65 (11.1%)
Missing	45

Our initial analysis involved determining the prevalence and pattern of FAK expression in our sample of invasive tumors. The characteristics of FAK expression, including percent tumors positive, intensity, and percent cells positive are presented in table 2.

Table 2. Characteristics of FAK expression in breast tumors

N = 629	
FAK expression (+/-)	
Positive	154 (24.5%)
Negative	475 (75.5%)
FAK intensity	
Mean	2.2
Median (range)	2.0 (0-4)
FAK Percent cells positive	
Mean	67.1%
Median (range)	80.0% (0-99%)

One of the goals of our study was to evaluate the correlation between FAK expression and severity of disease. Table 3 summarizes the prevalence and pattern of FAK expression in tumors stratified by stage at diagnosis. These data suggest that stage 2-4 tumors show greater FAK expression than stage I tumors, and that stage 2-4 tumors show higher FAK intensity and percent cells positive than stage I tumors, leading to the conclusion that FAK expression is associated with later stage at diagnosis.

Table 3. FAK expression by stage

N = 629 (45 of those missing stage at diagnosis)		
FAK expression (+/-)	% Positive	
Stage 1	19.8	
Stage 2	25.7	P>0.05
Stage 3+4	27.7	
FAK-intensity	Mean	Median (Range)
Stage 1	1.9	2.0 (0-4)
Stage 2	2.2	2.0 (0-4)
Stage 3+4	2.4	2.0 (0-4)
FAK-Percent cells positive	Mean	Median (Range)
Stage 1	62.6	80 (0-95)
Stage 2	68.7	80 (0-99)
Stage 3+4	70.1	90 (0-95)

Additionally we were interested in determining the association between FAK expression and invasive disease to ascertain whether FAK expression provides information independent of currently known risk and prognostic factors. Table 4 presents the association between FAK expression and other known risk factors in our sample. These data suggest that FAK+ tumors are strongly associated with HER2+ tumors and moderately associated with P53+ tumors. Additionally, FAK expression is

inversely associated with both ER+ and PR+ tumors. Furthermore, positive FAK expression is strongly associated with tumors with a high mitotic index and of high nuclear grade. There is evidence of an association with histologic grade. Lastly, the data suggest that FAK expression associated with positive lymph node status.

Table 4: Association of FAK expression with known risk factors.

Risk Factor	FAK Positive	FAK Negative	OR (95% CI)	Chisq p
P53 Status				
P53+	91	201	2.0(1.4-3.0)	P=0.0002
P53-	60	270		
HER2 Status				
HER2+	49	96	1.8(1.2-2.8)	P=0.003
HER2-	105	376		
Estrogen Receptor				
ER+	71	284	0.6(0.4-0.8)	P=0.002
ER-	78	173		
Progesterone Receptor				
PR+	65	275	0.5(0.3-0.8)	P=0.001
PR-	83	178		
Mitotic index				
High (MI>10)	93	180	2.5(1.7-3.7)	P=0.0001
Low (MI<=10)	59	290		
Histologic Grade				
Well-mod differentiated	44	173	1.4(1.0-2.2)	P=0.08
Poorly differentiated	109	299		
Nuclear Grade				
Slight-mod. Pleomorphism	57	308	3.1(2.1-4.7)	P<0.0001
Marked pleomorphism	96	165		
Lymph Node Status				
Positive	69	170	1.4(1.0-2.1)	P=0.05
Negative	79	281		

Once survival data has been collected, we will control for factors associated with FAK expression to determine if FAK expression provides information on prognosis independent of currently known risk and prognostic factors. Preliminary multivariate analysis evaluating the independent role of FAK as a predictor of lymph node metastasis suggests that FAK alone is not as good a predictor alone as HER2 alone, but both are better than P53. There is some evidence for an interaction between FAK+ and HER2+ status in predicting lymph node metastases as the joint presence of FAK+ and HER2+ is better than either marker alone (Table 5).

Table 5. OR's and 95% CI's for lymph node metastases based on FAK and HER2 expression status

Predictors	OR	95% CI	P-value
FAK+ / HER2-	1.2	0.8-2.0	0.37
FAK- / HER2+	1.7	1.0-3.0	0.04
FAK+ / HER2+	2.2	1.1-4.4	0.04

All OR's are adjusted for age, race, and menopausal status, and are mutually adjusted (OR's for FAK and HER2 are adjusted for P53 and vice versa)
All three OR's use the FAK-/HER2- as the referent group.

Key Research Accomplishments:

There are a number of key research accomplishments for the previous year of this project:

- We have developed a new, more thorough scoring system for E-cadherin, and that system has been reviewed by the pathologist serving as a consultant to the Carolina Breast Cancer study.
- Immunohistochemical staining of tumor tissue for E-cadherin expression is complete (n = ***).
- Immunohistochemical staining of tumor tissue for FAK expression is complete (n = 629).
- Scoring of FAK stained tissue is complete for all of the stained phase I cases.
- Scoring of E-cadherin stained tissue is complete.
- Data analysis for the FAK stained and scored samples has been performed.
- Data on E-cadherin expression is currently being entered into the database and preliminary data analysis for the E-cadherin stained and scored tissue should begin within the next two weeks.
- Application to the National Death Index has been completed and filed. We are awaiting approval and receipt of cause-specific cause of death for our phase I cases.

Reportable Outcomes:

Based upon the results from study on this project to date, an abstract and poster was presented at the 2002 DOD Era of Hope Breast Cancer Meeting in Orlando Florida. The poster was titled:

The role of E-cadherin and FAK expression as prognostic markers in breast cancer progression and survival

Sandra L. Deming[1], Amy L. Lark[2], Lynn G. Dressler[2], Dave W. Cowan[2], William G. Cance[2], and Robert C. Millikan[1,2]
[1] Department of Epidemiology, School of Public Health
[2] Lineberger Comprehensive Cancer Center
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Additionally, a presentation regarding this study was made at the Department of Epidemiology in the University of North Carolina's School of Public Heath in February 2003.

Conclusion: The conclusions based on the preliminary data indicate that the prevalence of FAK expression among invasive cases of Phase I of the Carolina Breast Cancer Study is 24.5%. FAK expression status was associated with positive P53 expression, positive Her2 expression, later stage at diagnosis, ER and PR negativity, high mitotic index, high nuclear grade (degree of pleomorphism), histologic grade (degree of differentiation), and lymph node metastasis. High FAK expression appears to be associated with known factors indicative of more aggressive disease and worse prognosis. Specifically, high FAK expressing tumors are 1.8 times more likely to have amplified Her2, 1.7 times more likely to be ER negative, 2.0 times more likely to be PR negative, 2.5 times more likely to have high mitotic activity, and 1.4 times more likely have lymph node involvement as compared to low expressers (all p-values < 0.05). Preliminary multivariate analysis evaluating the independent role of FAK as a predictor of lymph node metastasis suggests that FAK alone is not as good a predictor alone as HER2 alone. However, there appears to be an interaction between FAK+ and HER2+ in that the joint presence of FAK+ and HER2+ is better at predicting lymph node metastases than is either marker alone (OR=2.2, 95% CI=1.1-4.4). These data suggest that FAK+ in the context of HER2 status is a significant predictor of lymph node metastases.